Supplementary Information

Overexpression of PDGFRA cooperates with loss of NF1 and p53 to accelerate the molecular pathogenesis of malignant peripheral nerve sheath tumors

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Supplementary Materials and Methods

Paraffin Sectioning, Cryosectioning and Immunostaining

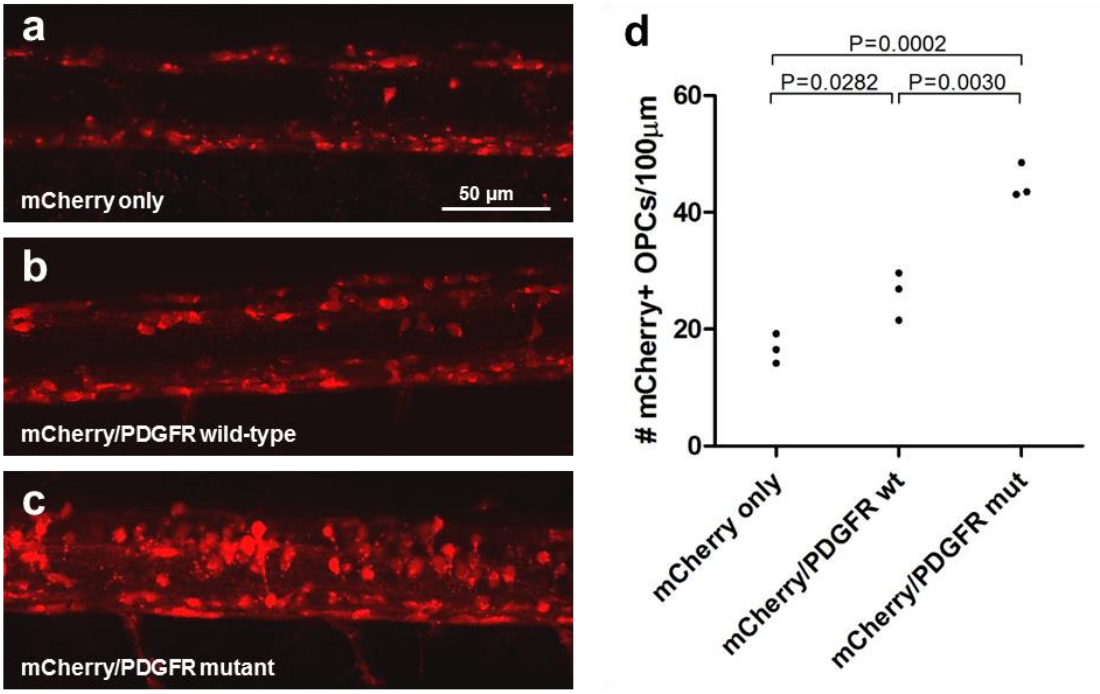
Fish were fixed with 4% paraformaldehyde and embedded in paraffin blocks for paraffin sectioning. Sections were stained by conventional protocols for Masson's trichrome stain. For endogenous zebrafish PDGFRA stain, paraffin sections were stained with PDGFRA (C-20) antibody (Santa Cruz sc-338; 1:1000). Cryosections were immunostained using antibodies against Collagen I (Abcam ab23730; 1:100), and CD31 (Abcam ab28364; 1:100). Alexa Fluor® 488 Goat Anti-Rabbit IgG (H+L) (Life Technologies A11034) used as a secondary antibody. Beta-gal stain with cryosectioned MPNST tumors was performed as described previously.

Embryonic implantation assay

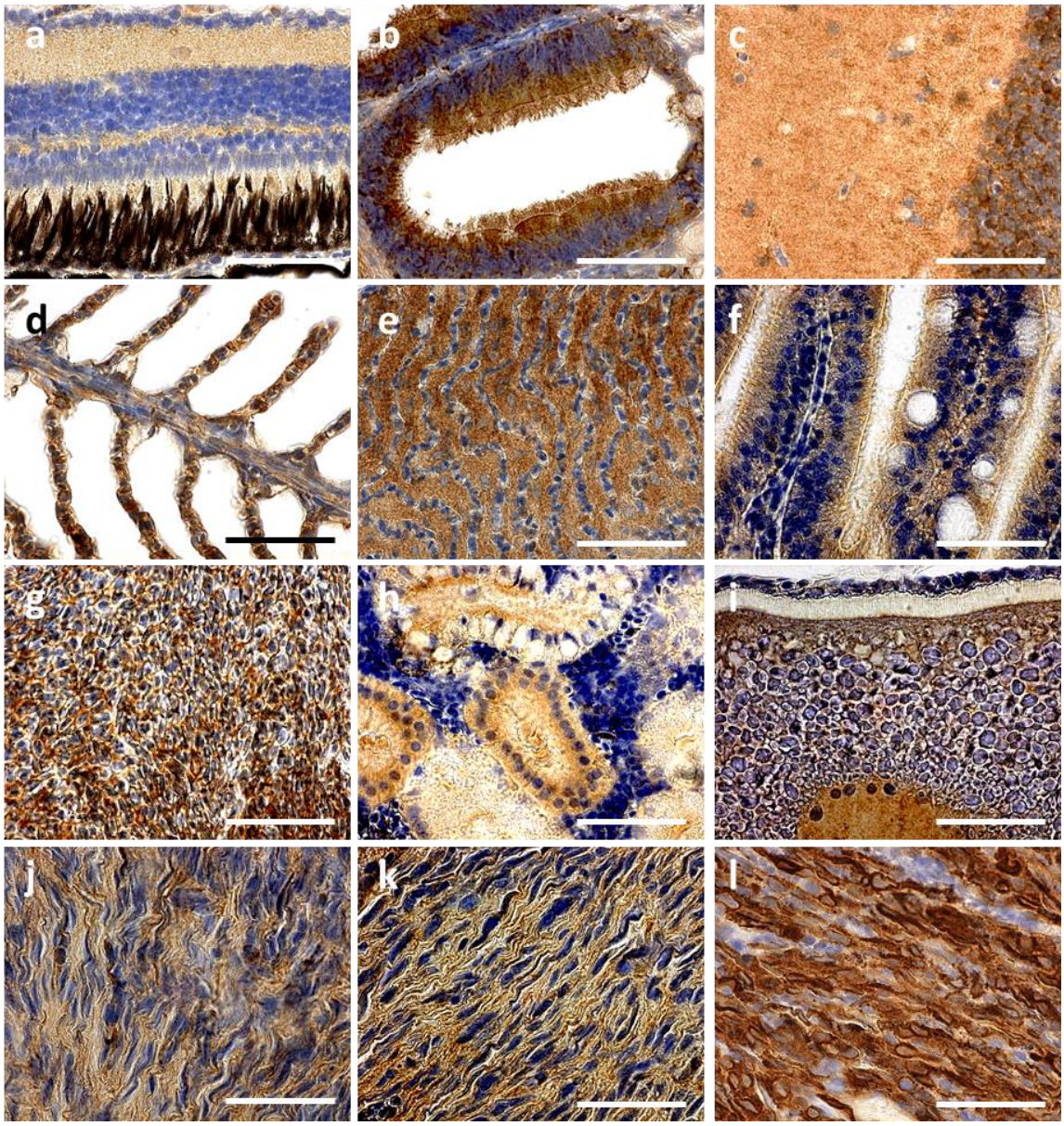
Pulled-capillary needles by the needle puller were sharpened with the micropipette grinder (EG-44, Narishige). The needle tip was opened using a tweezer, and then grinded for 1~2 minutes. Grind stone was sterilized with 70% ethanol before use. Grinding angle was set to 35 degrees using a protractor, and 70% ethanol was used as coolant during grinding. Casper (*roy* and *nacre* double homozygous mutant) embryos were collected and incubated at 28℃. Dechorionated embryos showing morphologically healthy and normal at 48 hpf were collected and used for embryonic implantation. Tumors were dissected from tumor-bearing fish under a fluorescent microscope after tricaine overdose treatment according to IACUC-approved protocols. The dissected tumors were manually disrupted using a blade with 2 ml of sterile suspension solution (0.9× PBS + 5% FBS, room temperature) until cloudy solution developed. The tumor cells and solution mixture was then filtered through a 35-μm cell strainer by gravity. The filtered cell solution was transferred to a sterile 1.5 ml tube, and then centrifuged with VWR Galaxy Mini Centrifuge C1413 for 3 minutes and supernatant removed. The tumor cell pellet was resuspended with the sterile suspension solution. Total number of viable cells was calculated by Trypan blue staining and hemocytometer counts before and after injection. 1.0~1.2 X 10^8 cells/ml concentration is good for injection. Two microliters of tumor cell suspension was loaded into a sharpened needle using Eppendorf microloader tips. Cell droplet size was measured with a micrometer, and set a diameter less than 120 micrometer (120 μm = about 1 nl) using the injection time and the pressure control knobs. Dechorionated embryos at 2dpf were anesthetized in egg water with tricaine, and transferred onto a 28 ℃ pre-warmed 10 cm injection plate (2% agarose). 1nl of MPNST cells (100~120 cells) were injected into the yolk sac of each embryo at 2dpf. Injection location was illustrated in Figure 7c. Implanted embryos were incubated in egg water at 28 ℃ for recovery. After 24 hours, embryos were screened under a fluorescent microscope, and successfully implanted embryos were collected for drug treatment. Twenty embryos were randomly distributed into a well of 12-well plate, and incubated in 1ml of egg water with either DMSO or drugs for 3 days at 28℃. Dead embryos were removed every day. After drug treatment for 3 days, each embryo was imaged, and tumor area was

quantitated using ImageJ software. The largest and smallest values of each condition were removed from analysis as outliers.

Supplementary Figures

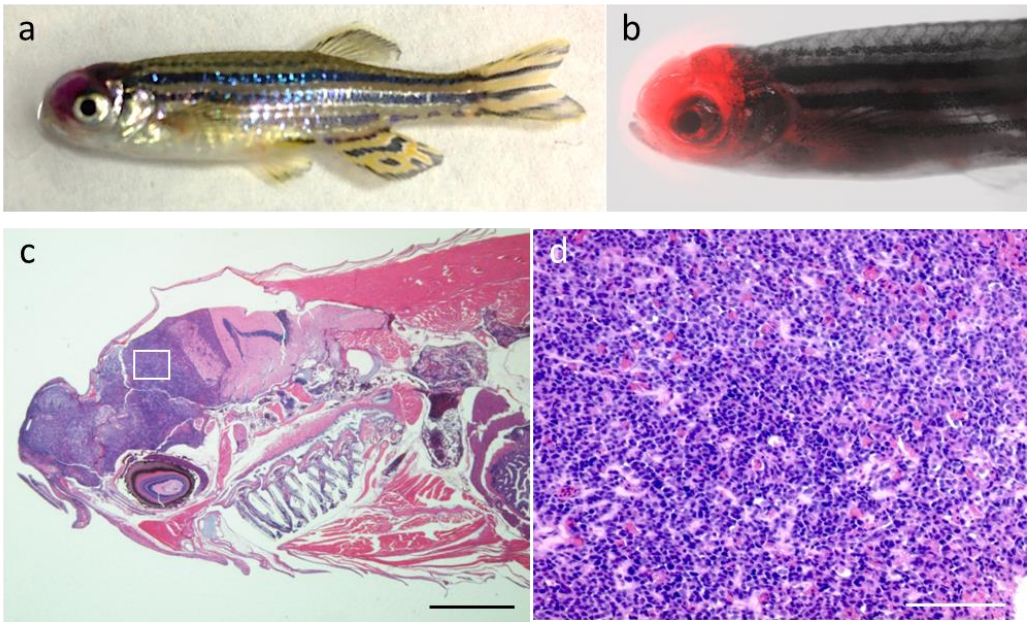


Supplementary Figure S1. PDGFRA overexpression causes hyperplasia of OPCs and Schwann cells (**a-c**) Representative confocal images of the spinal cords of mCherry control (n=3), wild-type PDGFRA (n=3), and mutant PDGFRA (n=3) transgenic 6 dpf larvae in the *nf1a*$^{+/+}$*; nf1b*$^{-/-}$*; p53*$^{m/m}$ background. Both (**b**) wild-type PDGFRA and (**c**) mutant PDGFRA transgenic larvae demonstrate increased numbers of sox10:mCherry-positive oligodendrocyte progenitor cells (OPCs) as compared to (**a**) mCherry control larvae in the *nf1a*$^{+/+}$*;nf1b*$^{-/-}$ *; p53* $^{m/m}$ background. (**d**) Quantification of the sox10:mCherry-positive OPC number within a 100 µm region of the thoracic spinal cord.

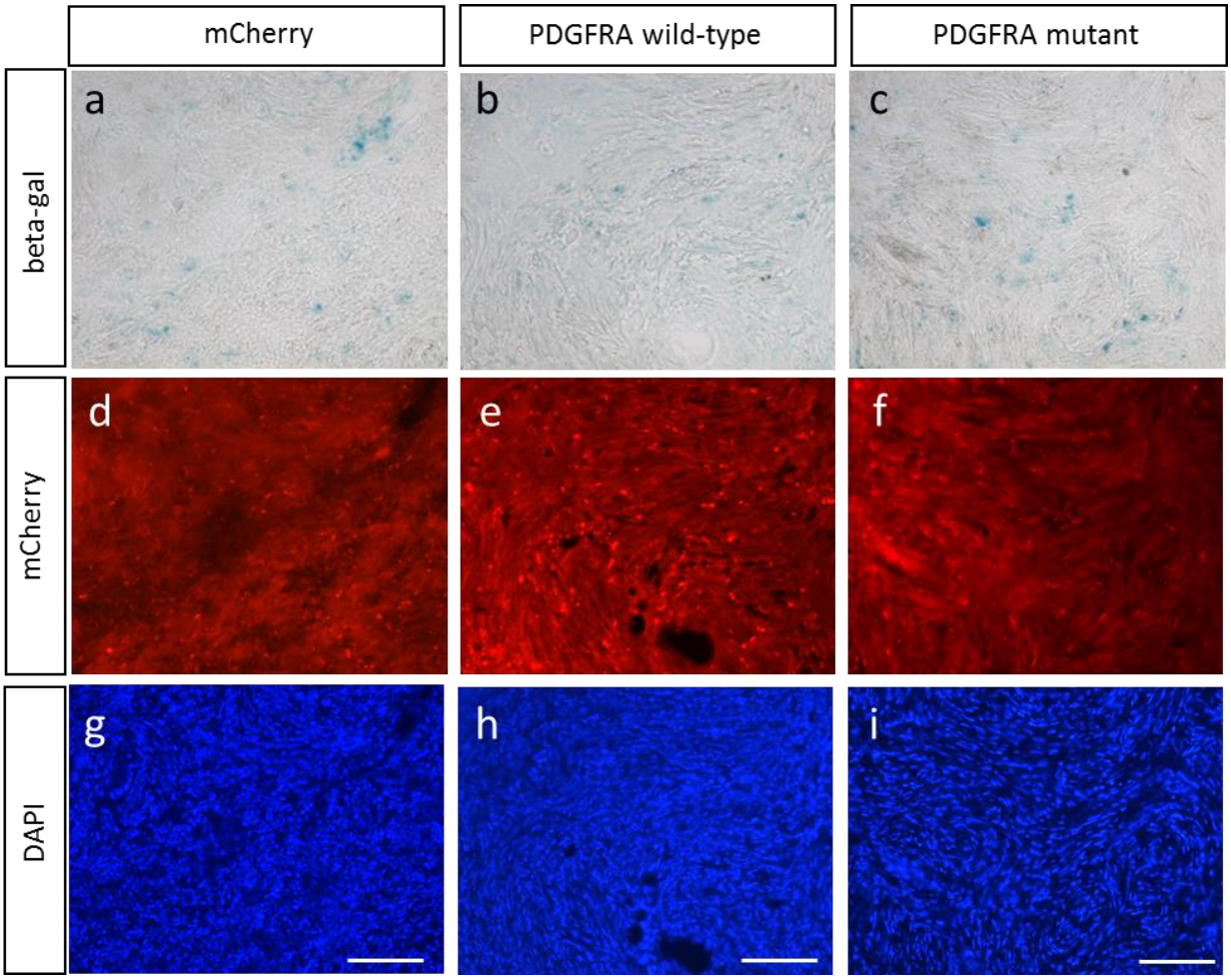


Supplemtary Figure S2. PDGFRA expressed tissues in adult fish

(**a-i**) Representative images of PDGFRA expression by immunohistochemistry in sox10:mCherry control transgenic zebrafish in the *nf1a*$^{+/-}$*;nf1b*$^{-/-}$*; p53*$^{m/m}$ background (>30 weeks post fertilization). PDGFRA is expressed in (**a**) the eye inner and outer plexiform layers, (**b**) olfactory ciliated columnar epithelium, (**c**) brain, (**d**) gill, (**e**) the gill pseudobranch, (**f**) intestine, (**g**) liver, (**h**) kidney, and (**i**) vitellogenic oocytes. (**j-l**) Representative images of PDGFRA expression by sox10:mCherry control (**j**), PDGFRA wild-type (**k**) and mutant MPNSTs (**l**) in the the *nf1a*$^{+/-}$*;nf1b*$^{-/-}$*; p53*$^{m/m}$ background, respectively (>30 weeks post fertilization, scale bar = 10 µm).

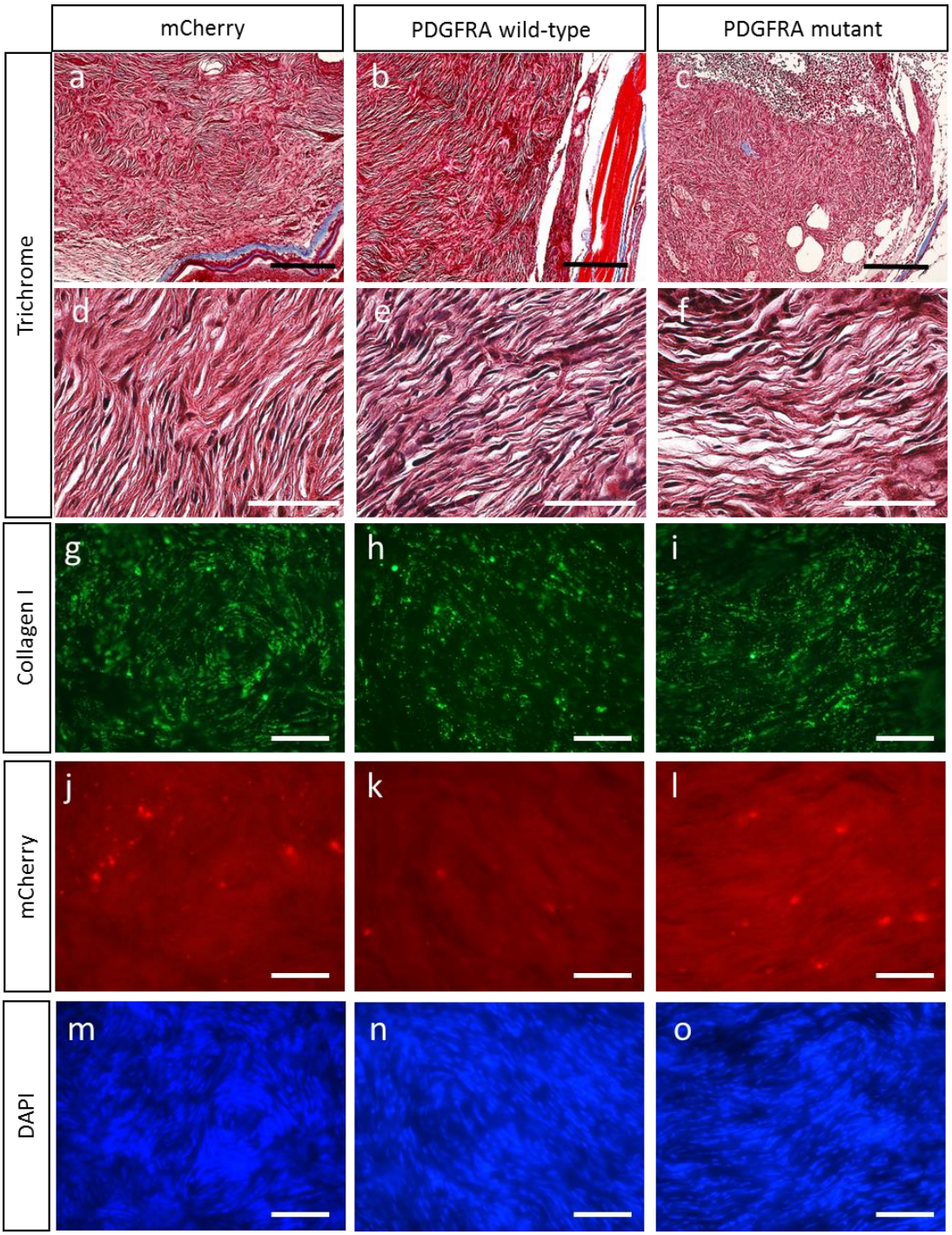


Supplemtary Figure S3. Images of the sox10 promoter driving mCherry-positive brain tumors
(**a**) A transgenic *sox10:mCherry/PDGFRA* transgenic fish haboring a brain tumor in the *nf1a*$^{+/-}$;*nf1b*$^{-/-}$; *p53* $^{m/m}$ background (>30 weeks post fertilization). (**b**) mCherry protein is strongly expressed in the glioma cells. (**c-d**) The histopathology after hematoxylin and eosin (H&E) staining of the zebrafish brain tumor. Panel **d** shows the glial tumor cells magnified from the white square area of panel **c**. (black scale bar = 1 mm, white scale bar = 0.1 mm).



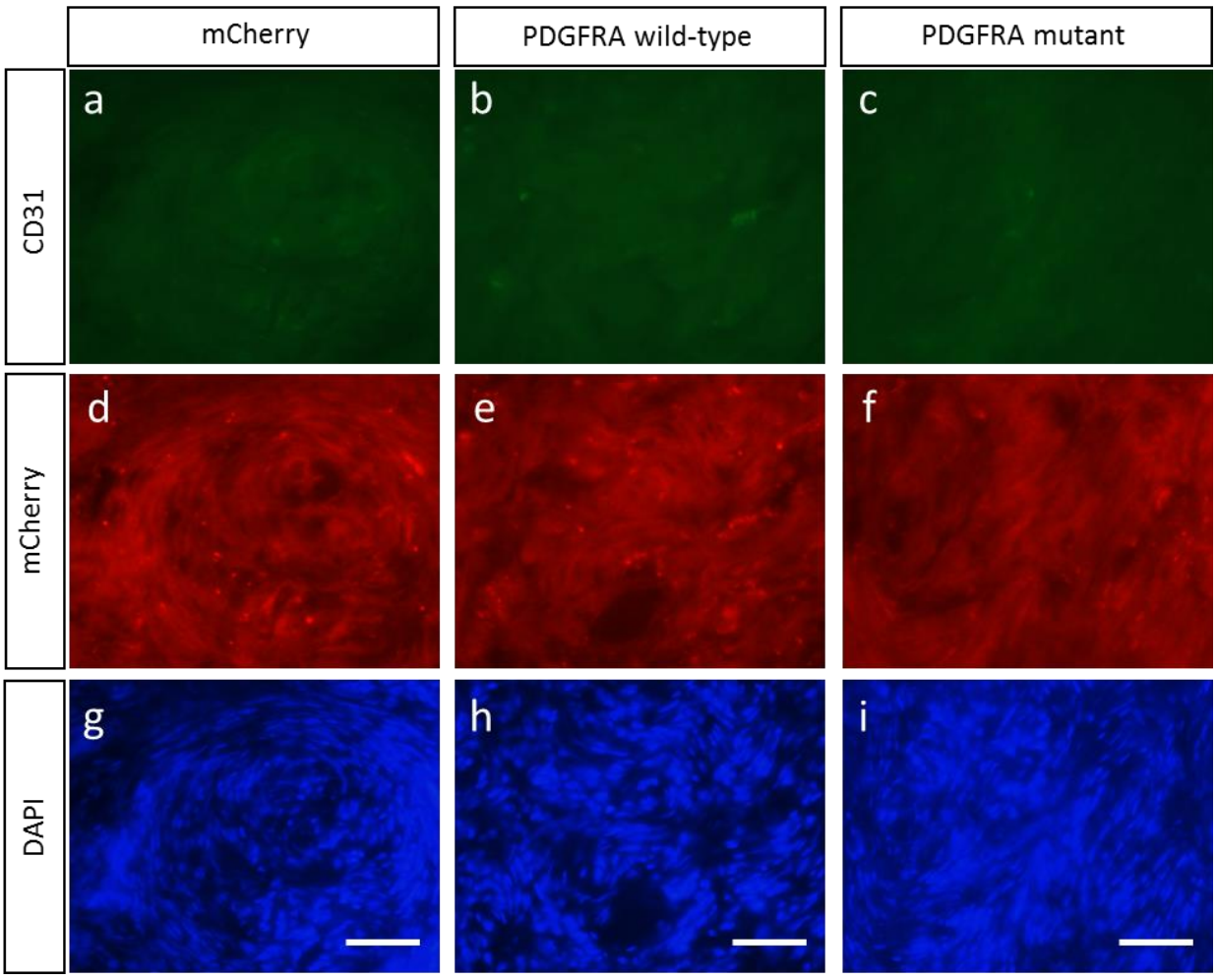
Supplementary Figure S4. Representative images of SA-β galactosidase stained-transgenic zebrafish MPNSTs

(**a-c**) Images of SA-β galactosidase stained-transgenic fish MPNSTs of (**a**) mCherry control, (**b**) PDGFRA wild type, and (**c**) PDGFRA mutant in the *nf1a+/-;nf1b-/- ; p53 m/m* background (>30 weeks post fertilization), with (**d-f**) mCherry and (**g-i**) DAPI (scale bar=100μm).



Supplementary Figure S5. Fibrosis of the transgenic zebrafish MPNSTs is not changed by either wild-type or mutant PDGFRA

(**a-c**) Representative images of the trichrome stained transgenic fish MPNSTs of (**a**) mCherry control, (**b**) PDGFRA wild type, and (**c**) PDGFRA mutant in the *nf1a*$^{+/-}$;*nf1b*$^{-/-}$; *p53* $^{m/m}$ background (>30 weeks post fertilization). Panels **d**, **e**, and **f** show the MPNST tumor cells magnified from panels **a**, **b**, and **c**, respectively.(**g-i**) Representative images of collagen I expression in transgenic fish MPNSTs of mCherry control with (**g-i**) mCherry and (**j-l**) DAPI (black scale bar=200 µm, white scale bar=10 µm).



Supplementary Figure S6. Vascularity of the induced MPNSTs is not altered by either wild-type or mutant PDGFRA (**a-c**) Representative images of CD31 expression in transgenic fish MPNSTs of (**a**) mCherry control, (**b**) PDGFRA wild type, and (**c**) PDGFRA mutant in the *nf1a*$^{+/-}$;*nf1b*$^{-/-}$; *p53* $^{m/m}$ background (>30 weeks post fertilization), with (**d-f**) mCherry and (**g-i**) DAPI (scale bar=10 µm).